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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR § 1.53 (b)(2).

Docket Number	959/6	Type a plus sign (+) inside this box->	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
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TITLE OF THE INVENTION (280 characters max)

TREATMENT OF CARDIAC FIBROSIS

CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

X	Specification	*Number of Pages*	22		*Small Entity Statement
X	Drawing(s)	*Number of Sheets*	3		Other (specify)

METHOD OF PAYMENT *(check one)*

	A check or money order is enclosed to cover the Provisional filing fees	PROVISIONAL FILING FEE AMOUNT($)	$150.00
X	The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number: 06-2140		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted,

SIGNATURE _____
TYPED or PRINTED NAME Mark M. FRIEDMAN

Date June 1, 1999
REGISTRATION NO. 33,883

☐ Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FILING ONLY

APPLICATION FOR PATENT

Inventor: Mark PINES

Title: TREATMENT OF CARDIAC FIBROSIS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to the treatment of cardiac fibrosis and, in particular, to the treatment of cardiac fibrosis with quinazolinone derivatives such as Halofuginone.

Cardiac fibrosis has a number of causes, which lead to the deposition of fibrotic tissue. As the deposition of such fibrotic tissue increases, the ability of the heart to function decreases, leading to disability and eventually death of the patient. The formation of fibrotic tissue in the heart is characterized by the deposition of abnormally large amounts of extracellular matrix components, including collagen, as well as other matrix proteins.

The synthesis of collagen is also involved in a number of other pathological conditions. For example, clinical conditions and disorders associated with primary or secondary fibrosis, such as systemic sclerosis, graft-versus-host disease (GVHD), lung fibrosis and a large variety of autoimmune disorders, are distinguished by excessive production of connective tissue, which results in the destruction of normal tissue architecture and function. These diseases can best be interpreted in terms of perturbations in cellular functions, a major manifestation of which is excessive collagen synthesis and deposition. The crucial role of collagen

in fibrosis has prompted attempts to develop drugs that inhibit its accumulation [K.I. Kivirikko, *Annals of Medicine*, Vol. 25, pp. 113-126 (1993)].

Such drugs can act by modulating the synthesis of the procollagen polypeptide chains, or by inhibiting specific post-translational events, which will lead either to reduced formation of extra-cellular collagen fibers or to an accumulation of fibers with altered properties. Unfortunately, only a few inhibitors of collagen synthesis are available, despite the importance of this protein in sustaining tissue integrity and its involvement in various disorders.

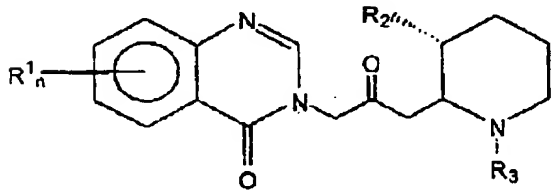
For example, cytotoxic drugs have been used in an attempt to slow the proliferation of collagen-producing fibroblasts [J.A. Casas, *et al., Ann. Rhem. Dis.*, **46**: 763, 1987], such as colchicine, which slows collagen secretion into the extracellular matrix [D. Kershenobich, *et al., N. Engl. J. Med.*, **318**:1709, 1988], as well as inhibitors of key collagen metabolism enzymes [K. Karvonen, *et al., J. Biol Chem.*, **265**: 8414, 1990; C.J. Cunliffe, *et al., J. Med. Chem.*, **35**:2652, 1992].

Unfortunately, none of these inhibitors are collagen-type specific. Also, there are serious concerns about the toxic consequences of interfering with biosynthesis of other vital collagenous molecules, such as Clq in the classical complement pathway, acetylcholine esterase of the neuro-muscular junction endplate, conglutinin and liver surfactant apoprotein.

Other drugs which can inhibit collagen synthesis, such as nifedipine and phenytoin, inhibit synthesis of other proteins as well, thereby non-specifically blocking the collagen biosynthetic pathway [T. Salo, *et al., J. Oral Pathol. Med.*, **19**: 404 ,1990].

Collagen cross-linking inhibitors, such as β-amino- propionitrile, are also non-specific, although they can serve as useful anti-fibrotic agents. Their prolonged use causes lathritic syndrome and interferes with elastogenesis, since elastin, another fibrous connective tissue protein, is also cross-linked. In addition, the collagen cross-linking inhibitory effect is secondary, and collagen overproduction has to precede its degradation by collagenase. Thus, a type-specific inhibitor of the synthesis of collagen itself is clearly required as an anti-fibrotic agent.

Such a type-specific collagen synthesis inhibitor is disclosed in U.S. Patent No. 5,449,678 for the treatment of a fibrotic condition. This specific inhibitor is a composition with a pharmaceutically effective amount of a pharmaceutically active compound of a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; R_3 is a member of the group consisting of

hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2. Pharmaceutically acceptable salts thereof are also included. Of this group of compounds, Halofuginone has been found to be particularly effective for such treatment.

U.S. Patent No. 5,449,678 discloses that these compounds are effective in the treatment of fibrotic conditions such as scleroderma and GVHD. WO Application No. 96/06616 further discloses that these compounds are effective in treating restenosis. The two former conditions are associated with excessive collagen deposition, which can be inhibited by Halofuginone. Restenosis is characterized by smooth muscle cell proliferation and extracellular matrix accumulation within the lumen of affected blood vessels in response to a vascular injury [Choi *et al., Arch. Surg*, **130**:257-261, 1995]. One hallmark of such smooth muscle cell proliferation is a phenotypic alteration, from the normal contractile phenotype to a synthetic one. Type I collagen has been shown to support such a phenotypic alteration, which can be blocked by Halofuginone [Choi *et al*, *Arch. Surg.*, **130**: 257-261, 1995; U.S. Patent No. 5,449,678].

However, the process of restenosis differs from cardiac fibrosis. Furthermore, cardiac tissue is generally different than the tissue of other organs. In particular, cardiac tissue must maintain the ability to function as a single muscle according to a wave of electrical activity in order to pump blood effectively. Therefore, the heart must maintain a high level of functionality, in contrast to an organ such as the liver for example, which can be significantly compromised and still provide the required level of function in order to maintain the body. Thus, any amount of cardiac fibrosis is detrimental to the functioning of the heart, such

that treatments which would be suitable for other organs would not be expected to be suitable for treating and/or preventing cardiac fibrosis.

In addition, the *in vitro* action of Halofuginone does not always predict its *in vivo* effects. For example, Halofuginone inhibits the synthesis of collagen type I in bone chrondrocytes *in vitro*, as demonstrated in U.S. Patent No. 5,449,678. However, chickens treated with Halofuginone were not reported to have an increased rate of bone breakage, indicating that the effect is not seen *in vivo*. Thus, the exact behavior of Halofuginone *in vivo* cannot always be accurately predicted from *in vitro* studies.

Furthermore, the ability of Halofuginone or other related quinazolinones to block or inhibit pathological processes related to cardiac fibrosis has not been demonstrated. For the reasons mentioned earlier, such an ability would not be expected or predicted from the ability of Halofuginone to treat other fibrotic conditions.

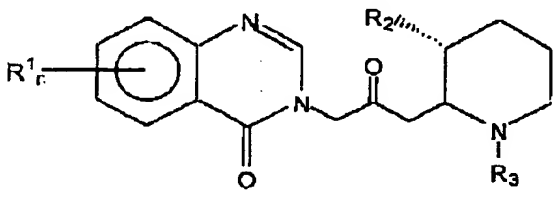
Thus, simply administering known *in vitro* inhibitors of collagen synthesis, deposition and cross-linking in an attempt to treat cardiac fibrosis is ineffective. Clearly, new treatments for this incurable disease are required which specifically slow or halt the pathogenesis of fibrosis, without non-specific or toxic side effects.

There is thus a widely recognized need for, and it would be highly advantageous to have, a treatment for cardiac fibrosis which inhibits fibrogenesis substantially without undesirable non-specific or toxic side effects.

SUMMARY OF THE INVENTION

Unexpectedly, it has been found, as described in the examples below, that Halofuginone can also inhibit the pathophysiological process of cardiac fibrosis *in vivo*, possibly by inhibiting collagen type I synthesis, although another mechanism or mechanisms could also be responsible. While inhibition of collagen type I synthesis is proposed as a plausible mechanism, it is not desired to be limited to a single mechanism, nor is it necessary since the *in vivo* data presented below clearly demonstrate the efficacy of Halofuginone as an inhibitor of cardiac fibrosis *in vivo*.

According to the teachings of the present invention, there is provided a composition for treating cardiac fibrosis, including a pharmaceutically effective amount of a compound in combination with a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:

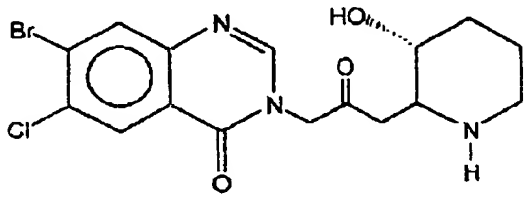


wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; R_3 is a member of the group consisting of

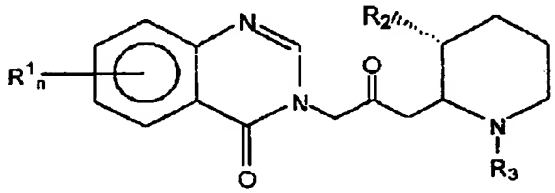
hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2. Pharmaceutically acceptable salts thereof are also included.

According to further preferred embodiments of the present invention, the compound is preferably Halofuginone. Hereinafter, the term "Halofuginone" is defined as a compound having a formula:



and pharmaceutically acceptable salts thereof. The composition preferably includes a pharmaceutically acceptable carrier for the compound.

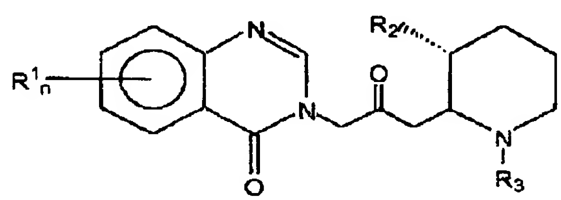
According to another embodiment of the present invention, there is provided a method of manufacturing a medicament for treating cardiac fibrosis, including the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy, R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2. Pharmaceutically acceptable salts thereof are also included.

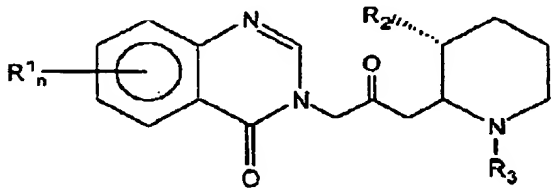
According to yet another embodiment of the present invention, there is provided a method for the treatment of cardiac fibrosis in a subject, including the step of administering a pharmaceutically effective amount of a compound having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2. Pharmaceutically acceptable salts thereof are also included.

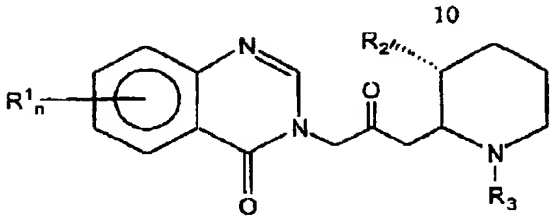
According to other embodiments of the present invention, there is provided a composition for substantially preventing the genesis of cardiac fibrosis, including a pharmaceutically effective amount of a compound in combination with a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and n is either 1 or 2. Pharmaceutically acceptable salts thereof are also included.

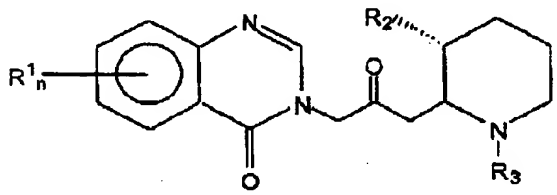
According to still other embodiments of the present invention, there is provided a method of manufacturing a medicament for substantially preventing the genesis of cardiac fibrosis, including the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2. Pharmaceutically acceptable salts thereof are also included.

According to still other embodiments of the present invention, there is provided a method for substantially preventing the genesis of cardiac fibrosis in a subject, including the step of administering a pharmaceutically effective amount of a compound having a formula:

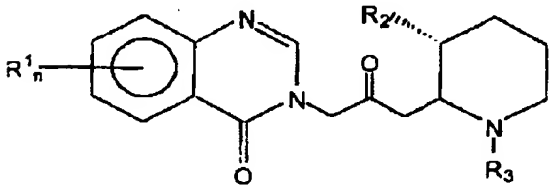


wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2. Pharmaceutically acceptable salts thereof are also included.

Hereinafter, the term "subject" refers to the human or lower animal to whom Halofuginone was administered. The term "patient" refers to human subjects. The term "treatment" includes both substantially preventing the process of cardiac fibrosis from starting and slowing or halting the progression of cardiac fibrosis once it has arisen. The phrase "substantially preventing the genesis" of cardiac fibrosis is understood to refer to the prevention of the appearance of clinical or preclinical symptoms of these conditions.

Although the specific quinazolinone derivative "Halofuginone" is referred to throughout the specification, it is understood that other quinazolinone derivatives may be used in its place, these derivatives having the formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy; R_2 is a member of the group consisting of

hydroxy, acetoxy and lower alkoxy, R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2. Pharmaceutically acceptable salts thereof are also included.

While the invention will now be described in connection with certain preferred embodiments in the following figures and examples so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following figures and examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of formulation procedures as well as of the principles and conceptual aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1 illustrates the effect of Halofuginone on ventricular collagen volume fraction (CVF) in rat heart;

FIG. 2 illustrates the effect of Halofuginone on collagen α1(I) gene expression in rat heart; and

FIG. 3 illustrates the effect of Halofuginone on TGF-β expression in rat heart.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unexpectedly, it has been found, as described in the examples below, that Halofuginone can inhibit the pathological process of cardiac fibrosis *in vivo*, possibly by inhibiting collagen type I synthesis, although another mechanism or mechanisms could also be responsible. Indeed, irrespective of the specific mechanism, the data presented below clearly demonstrate the efficacy of Halofuginone *in vivo* for inhibition of the pathological progression of cardiac fibrosis.

Such a finding is unexpected for three reasons. First, the behavior of Halofuginone *in vitro* does not exactly correspond to its behavior *in vivo*. This can be demonstrated by the differential effect of Halofuginone observed with bone chondrocytes *in vivo* and *in vitro*. Halofuginone inhibits the synthesis of collagen type I in chrondrocytes *in vitro*, as demonstrated in U.S. Patent No. 5,449,678. However, chickens treated with Halofuginone were not reported to have an increased rate of bone breakage, indicating that the effect is not seen *in vivo*. Thus, the exact behavior of Halofuginone *in vivo* cannot always be accurately predicted from *in vitro* studies.

Second, Halofuginone has only been shown to be a collagen type I inhibitor. However, the formation of fibrotic tissue in the heart is characterized by the deposition of abnormally large amounts of extracellular matrix components. Thus, the ability of Halofuginone to inhibit collagen type I synthesis and deposition cannot predict the ability of Halofuginone to slow, reduce or other ameliorate the pathogenesis of cardiac fibrosis.

Third, as demonstrated below, Halofuginone is able to prevent cardiac fibrosis by inhibiting the deposition of type I collagen, without downregulating or otherwise altering the synthesis of TGF β (transforming growth factor), which is a cytokine generally affecting the synthesis and deposition of several ECM components. Without wishing to be limited by a single mechanism, Halofuginone may be exerting its effect through an influence on type I collagen transcription. Thus, the effects of Halofuginone are specific and restricted, yet are able to prevent cardiac fibrosis.

Thus, nothing in the prior art taught that Halofuginone would be useful in the treatment of cardiac fibrosis *in vivo*. Furthermore, the ability of Halofuginone, and related compounds, to slow or halt progression of fibrosis in the heart is both novel and non-obvious. The demonstration of such an ability *in vivo* is particularly unexpected, given the differential responses seen *in vitro* and *in vivo* to Halofuginone.

The present invention may be more readily understood with reference to the following illustrative examples and figures. It should be noted that although reference is made exclusively to Halofuginone, it is believed that the other

quinazolinone derivatives described and claimed in U.S. Patent 3,320,124, the teachings of which are incorporated herein by reference, have similar properties.

The present invention is of a treatment for cardiac fibrosis with quinazolinone-containing compounds such as Halofuginone. Both compositions with specific pharmaceutical formulations and methods of using these compounds are described below.

Although the pathogenesis of cardiac fibrosis is not fully understood, animal models for the disease have been successfully developed. Cardiac fibrosis has been induced in rats by the chronic administration of angiotensin II (Ang II).

Compounds which are intended for the inhibition of cardiac fibrosis must be tested in an *in vivo* model, such as the Ang II model described above, for their ability to slow or halt the pathological process leading to deposition of fibrotic tissue. Such experiments were conducted for the collagen type I synthesis inhibitor Halofuginone, as described in greater detail in Example 1 below.

Furthermore, once demonstrably effective compounds have been discovered, specific formulations and routes of administration must be elucidated for maximum efficacy of the treatment. Such formulations and routes of administration must enable the compound to be effectively absorbed and delivered to the desired site of treatment, while minimizing non-specific side effects caused by systemic distribution of the compound. Illustrative examples of these formulations and routes of administration for quinazolinone-containing compounds such as Halofuginone are given in Example 2 below.

Example 1

Effect of Halofuginone on Histology and Morphology of Rat Heart

Histological examination of heart samples from control and Ang II (angiotensin II)-treated rats revealed that Ang II induced specific morphological changes in rat heart, including increased collagen fiber content. Halofuginone substantially inhibited the occurrence of these morphological changes, resulting in rat heart of more normal appearance.

The experimental method was as follows. Male Sprague-Dawley rats were divided into four groups. Two groups were chronically infused with Ang II at the rate of 0.150 ng/min by implanted mini-pump. This dosage regimen will induce severe cardiac fibrosis. The other two groups of rats, control rats, were injected with saline. One group of Ang II-treated rats and one control group were daily injected intraperitoneally with 16 micrograms of Halofuginone. At the end of the experimental period, the rats were sacrificed and the heart was removed and weighed.

Heart samples were taken for histological examination. Briefly, the tissue samples were collected into phosphate-buffered saline (PBS) and fixed overnight in 4% paraformaldehyde in PBS at 4 °C. Serial 5 μm sections were prepared after the samples had been dehydrated in graded ethanol solutions, cleared in chloroform and embedded in Paraplast. Differential staining of collagenous and non-collagenous proteins was performed with 0.1% Sirius red and 0.1% fast green as a counter-stain in picric acid. This procedure stains collagen red [Gascon-Barre, M., *et al.*, *J. Histochem. Cytochem.*, 37:377-381, 1989].

Heart samples were then hybridized with a probe for rat collagen α1(I) expression, or with a probe for TGF β1 expression. For hybridization with one of the genetic probes, the sections were deparafinized in xylene, rehydrated through a graded series of ethanol solutions, rinsed in distilled water for 5 minutes and then incubated in 2X SSC at 70 °C for 30 minutes. The sections were then rinsed in distilled water and treated with pronase, 0.125 mg/ml in 50 mM Tris-HCl, 5 mM EDTA, pH 7.5, for 10 minutes. After digestion, the slides were rinsed with distilled water, post-fixed in 10% formalin in PBS and blocked in 0.2% glycine. After blocking, the slides were rinsed in distilled water, rapidly dehydrated through graded ethanol solutions and air-dried for several hours. Before hybridization, the 1600 bp rat collagen α1(I) insert was cut out from the original plasmid, pUC18, and inserted into the pSafyre plasmid. The sections were then hybridized with this probe after digoxigenin-labeling [M. Pines *et al.*, *Matrix Biology*, 14:765-71, 1996]. Similarly, other slides were hybridized with a probe for TGF-β1.

Figure 1 shows the result of collagen volume quantitation of rat heart after videodensitometry. Sections of rat liver tissue were stained with Sirius red to demonstrate collagen content of the tissue. A low volume of collagen was observed in control rats (CON) and rats which received Halofuginone alone (HAL). A high volume of collagen was observed in rats which received Ang II (A II) alone, but was markedly reduced in rats given both Ang II and Halofuginone (A II + HAL), indicating the ability of Halofuginone to substantially inhibit the pathophysiological process of fibrosis induced by Ang II. Indeed, the ventricular

collagen volume fraction (CVF) was increased by threefold ($p<0.05$) in rats treated with Ang II, compared to rats treated with either Halofuginone alone or Halofuginone plus Ang II. In addition, the ventricular CVF for control rats was identical to that for rats treated with Halofuginone alone. Indeed, over a two week period, Halofuginone did not alter the ventricular CVF when administered alone, without Ang II. Thus, Halofuginone had a strong ability to prevent the increase in ventricular CVF which is induced by Ang II, without having effect alone on ventricular CVF.

Figure 2 shows the results of densitometery measurements after *in situ* hybridization of a section of rat heart tissue with rat collagen α1(I) probe. A low expression of collagen α1(I) gene is seen in heart tissue of rats given Halofuginone alone (HALO). A marked increase in the expression of collagen α 1(I) gene was seen in the liver of rats given Ang II alone (Ang II). Rats given both Halofuginone and Ang II show a marked reduction in the expression of collagen α 1(I) gene (Ang II + HALO), as compared to rats given Ang II alone. Although this dose of Halofuginone substantially reduced the increase in rat collagen α1(I) gene expression caused by Ang II, it did not completely inhibit such expression. However, the substantially reduced rat collagen α1(I) gene expression indicates that Halofuginone is effective against the pathological induction of expression by Ang II. Thus, the five fold rise in type I collagen mRNA level ($p<0.05$), induced by Ang II, was attenuated by Halofuginone.

Figure 3 shows that although Ang II caused an increase in the expression of mRNA for TGF β ($p<0.05$), this enhancement was not affected by the

administration of Halofuginone. In particular, the level of TGF β expression was significantly and equally higher in rats given either Ang II (AngII) or Ang II plus Halofuginone (Ang II + HALO), than in rats given Halofuginone alone (HALO). Thus, Halofuginone does not appear to inhibit the processes of extracellular matrix synthesis and deposition which are controlled by TGF β.

Thus, Halofuginone is able to prevent cardiac fibrosis by inhibiting the deposition of type I collagen, without altering the synthesis of TGF β, which is a cytokine affecting the synthesis and deposition of several ECM components. The effects of Halofuginone appear to be specific to a particular aspect of the pathway for extracellular matrix synthesis, which is neither taught nor suggested by the background art. Therefore, the specificity of Halofuginone appears to be directed to a particular mechanism of collagen synthesis and deposition, a mechanism which does not appear to be controlled by TGF β.

Overall, Halofuginone was able to prevent the appearance of the effects of Ang II-induced fibrosis on all levels, including marked reduction of gross and fine morphological changes caused by Ang II-induced fibrosis. Clearly, the effects of Halofuginone are both potent and specific for the prevention of the morphological changes produced during the pathological process of cardiac fibrosis.

<u>Example 2</u>
<u>Suitable Formulations for</u>
<u>Administration of Halofuginone</u>

Halofuginone can be administered to a subject in a number of ways, which are well known in the art. Hereinafter, the term "subject" refers to the human or

lower animal to whom Halofuginone was administered. For example, administration may be done orally, or parenterally, for example by intravenous drip or intraperitoneal, subcutaneous, or intramuscular injection.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

Formulations for parenteral administration may include but are not limited to sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Dosing is dependent on the severity of the symptoms and on the responsiveness of the subject to Halofuginone. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

<u>Example 3</u>
<u>Method of Treatment of Cardiac Fibrosis</u>

As noted above, Halofuginone has been shown to be an effective inhibitor of cardiac fibrosis. The following example is an illustration only of a method of treating cardiac fibrosis with Halofuginone, and is not intended to be limiting.

The method includes the step of administering Halofuginone, in a pharmaceutically acceptable carrier as described in Example 2 above, to a subject to be treated. Halofuginone is administered according to an effective dosing methodology, preferably until a predefined endpoint is reached, such as the

absence of further progression of cardiac fibrosis in the subject, the inhibition of cardiac fibrosis or the prevention of the formation of cardiac fibrosis.

Example 4
Method of Manufacture of
a Medicament Containing Halofuginone

The following is an example of a method of manufacturing Halofuginone. First, Halofuginone is synthesized in accordance with good pharmaceutical manufacturing practice. Examples of methods of synthesizing Halofuginone, and related quinazolinone derivatives, are given in U.S. Patent No. 3,338,909. Next, Halofuginone is placed in a suitable pharmaceutical carrier, as described in Example 2 above, again in accordance with good pharmaceutical manufacturing practice.

It will be appreciated that the above descriptions are intended only to serve as examples, and that many other embodiments are possible within the spirit and the scope of the present invention.

ABSTRACT OF THE DISCLOSURE

A composition for treating cardiac fibrosis and a method of using and manufacturing the composition are provided. The composition includes a quinazolinone derivative, preferably Halofuginone.

Collagen Volume Quantitation in the Rat Heart

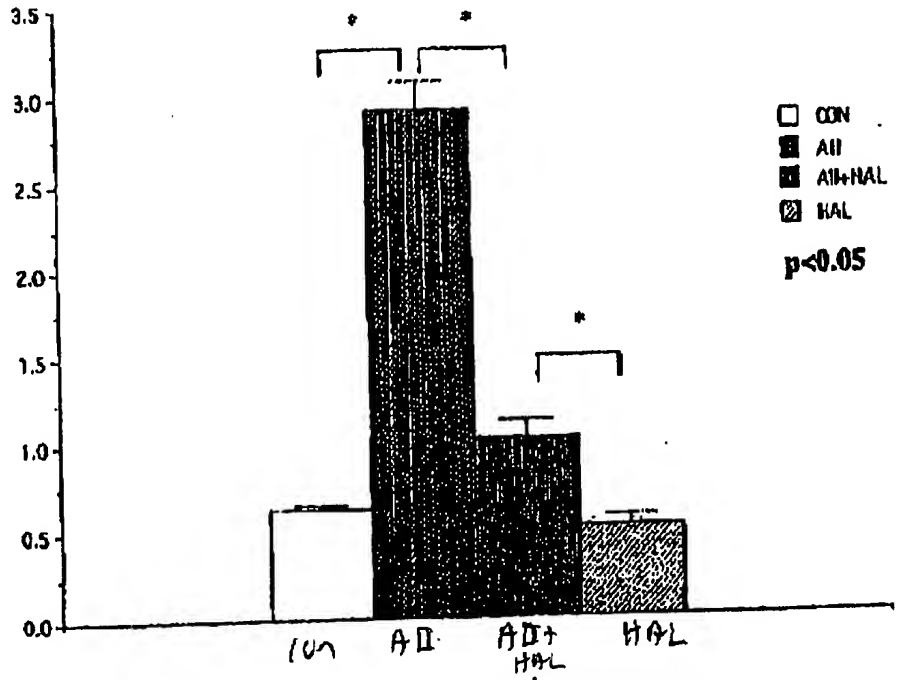


Figure 1

Figure 2

Figure 3